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EFFECTS OF GAMMA RADIATION ON REPRODUCTION, MATING COMPETITIVENESS AND SPERM ACTIVITY OF *CALLOSOBRUCHUS CHINENSIS* (L.)

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تأثير أشعة جاما على التكاثر والتنافس التزاوجي ونشاط الحيوان المنوي لحشرة *كالوسوبروكس تشيننسيس*

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تمت دراسة تأثير الجرعات المختلفة لأشعة جاما (٤٠ ، ٨٠ ، ١٢٠ جراى) على الطور اليافع لخنفساء *كالوسوبروكس تشيننسيس* وتبين أن كمية البيض وخصوبته تقل بزيادة الجرعة الاشعاعية . وقد كانت التأثيرات أكثر حدة في حالة تزاوج كلا من الجنسين المعاملين عما لو كان أحدهما هو المعامل ، وقد لوحظ أن الجرعة المسببة للعقم لكل من الذكور والإناث هي ١٢٠ جراي .

كما لوحظ أن النسبة المئوية للعقم في الحشرات المشعة لم تقل بتجزئة الجرعة المعقمة . كذلك لم يوجد تأثير واضح على كمية ما تضعه الأنثى من البيض وفترة حياة الحشرات وكما أظهرت الذكور المشعة قدرة فائقة على التنافس التزاوجي مع الذكور غير المشعة في كل الجرعات والنسب المستعملة . كذلك أوضحت نتائج التجربة التي أجريت لدراسة كفاءة الحيوانات المنوية للذكور المشعة بالجرعة المسببة للعقم (١٢٠ جراى) أن الحيوانات المنوية للذكور المشعة كان لها القدرة على التنافس التزاوجي مع الحيوانات المنوية للذكور غير المعاملة بالاشعاع .

ABSTRACT

The effects of gamma irradiation at different doses on the adult stage of *Callosobruchus chinensis* L. were studied. Adult fecundity and fertility decreased by increasing the radiation dose and effects of the treatment were more severe when both sexes were irradiated and mated together than matings of either sex with unirradiated partner. The dose required to sterilize males and females was about the same: 120 Gy was sterilizing to both sexes. Fractionation of sterilizing dose did not reduce perrcent sterility of irradiated adults, and there is no clear effect on fecundity or adult longevity. Irradiated males were full sexually competitive with unirradiated males at all doses of ratios used. Males irradiated with a sterilizing dose (120 Gy) were able to negate previous insemination by unirradiated ones, sperms of the sterile males could compete with sperms of the normal males. These results indicated that sterile males give good results in controlling a population of *Callosobruchus chinensis* in an autocidal control programe.

INTRODUCTION

The pulse beetle, *Callosobruchus chinensis* (L.) is one of the most destructive pests of stored leguminous in Egypt, such as, peas, cowpeas, beans and lentils. This bruchid beetle has become of world-wide distribution.

The use of gamma radiation to control stored-product insects in bulk grain is technically feasible. Control can be obtained with lower, more economical doses of radiation if the resident insect population are sterilized rather than killed.

One of the criteria for success of this technique is gamma sterilized males compete favourably with normal males. Cornwell *et al.* [1] suggested that the presence of sterile but sexually competitive insects might offer some measure of protection against reinfestation.

Production of insects that are both sterile and sexually competitive is indispensible to a successful programe involving release of sterile males for suppression of a field population.

The aim of present investigation is concerned with determining the sterilizing dose of gamma radiation for male and female beetles then given as two or three equal fractions with certain time intervals between each fraction. Furthermore, the effects of sterilizing and substerilizing doses on mating competitiveness of males and sperm activity of radiosterilized males.

MATERIAL AND METHODS

Stock cultures of *C. chinensis* were obtained from laboratory stock cultures and maintained at 27 ± 1°C and 65 ± 5% R.H. in three liter jars on sterilized lentil seeds. To obtain newly emerged unmated adults, the technique described by Haiba [2] was used. Newly emerged unmated adults were irradiated by CO^{60} gamma cell installed at the Atomic Energy Authority, Cairo, Egypt, at dose rate of 3.1 rad/sec.

The first experiment was made to determine the effects of gamma irradiation on fecundity and fertility of both sexes. Four pairing combinations were used for each dose, as follows: irradiated males X unirradiated females Io X Uo, Uo X Io, Io X Io and Uo X Uo as a control group. Newly emerged unmated adults were irradiated at 40, 80 and 120 Gray. After irradiation, pairs of adults were placed singly in tubes 2 X 1 inch on 100 lentil seeds as an oviposition sites. Ten tubes were set up for each dose, and repeated 5 times.

The second experiment was to study the effects of the sterilizing dose applied as acute dose or divided into two or three equal fractions with 5 hours intervals between each fraction. Records were taken for adult fecundity, fertility and longevity of both sexes.

The third experiment was to determine sexual competitiveness of males irradiated at sterilizing and substerilizing doses. Immediately after treatment, the irradiated males at each dose were caged with unirradiated males and females at different ratios, i.e. 1:1:1, 5:1:1 and 10:1:1 (Io : Uo : Uo). The numbers of hatched eggs were recorded. The experiment was replicated five times. The mathematical method for calculating the competitiveness value (C.V.) of irradiated males described by Fried [3] was used.

$$C.V. = \frac{Ha - Hob}{Hob - HS} - \frac{S}{N}$$

Ha = % hatchability of Uo X Uo

Hob = % hatchability of Io X Uo X Uo

HS = % hatchability of Io X Uo

S = No. of sterile males.

N = No. of fertile males.

The fourth experiment was conducted to determine the effects of gamma irradiation on sperm activity of irradiated males. Soon after emergance, males were irradiated at 120 Gray (a sterilizing dose). A combination of matings between 5 unirradiated (U) males, and 5 unirradiated virgin females was made and left for 2 days, then the (U) males were replaced by 5 irradiated (I) males. Another combination of matings between 5(I) males with 5(U) virgin females were left for 2 days, then (I) males were replaced by 5(U) males. Egg production and egg hatchability were recorded throughout the first 2 days and throughout the next 2 days after replacement. Five replications from each combination were made.

RESULTS AND DISCUSSION

Data in Table (1) shows that in all mating combinations, fecundity was affected with all doses used. Increasing the dose decreased adult fecundity. For example, when irradiated females at 40, 80 and 120 Gray mated with unirradiated males, the average number of eggs per female were 46.2, 38.0 and 25.7, respectively, as compared with 58.8 in the control group. The greater reduction in fecundity was obtained when both sexes were irradiated and mated together. Adult irradiation reduced fertility and the reduction was correlated with the dose. The sterilizing dose for males or females paired with the untreated unmated opposite sex was 120 Gray, while, it decreased to 80 Gray when both sexes were irradiated and mated together. The effect of gamma

Table 1

Effects of gamma radiation and type of mating on fecundity and percentage of sterility of *C. chinensis* irradiated as newly emerged unmated adults.

Dose (Gray)	Mating Combination			Av. No. of Eggs per Female	% Sterility
	Male		Female		
0	U	x	U	58.8	12.2
40	I	x	U	52.1	84.3
	U	x	I	46.2	90.0
	I	x	I	40.6	93.3
80	I	x	U	41.2	96.5
	U	x	I	38.0	98.8
	I	x	I	27.8	100.0
120	I	x	U	31.5	100.0
	U	x	I	25.7	100.0
	I	x	I	12.3	100.0

I = Irradiated U = Unirradiated Av. = Average

radiation on fecundity and fertility was demonstrated by Ahmed [4] on *Callosobruchus maculatus* found that egg production was adversely affected as a result of adult irradiation, the higher the dose the lower the fecundity. The percentages of sterility increased gradually by increasing the dose to reach 100% at 100 Gray, when both sexes were treated at 150 Gray when the males only were treated. Study of Brower [5] showed that there were no differences in sterilization effect between males of *Tribolium destructor* irradiated with gamma radiation, both males and females were sterilized with 100 Gray. Investigation of Haiba [2] on newly emerged adults of *Callosobruchus chinensis* showed that the adult fecundity and fertility were progressively reduced as the dose level increased. This effect was more pronounced when irradiated females were crossed with irradiated males than when irradiated males or females crossed with unirradiated opposite sex, the dose 90 Gray caused 100% sterility in males or females while only the dose 60 Gray gave the same effect when both sexes were irradiated and mated together. Hussain and Lmura [6] found that the fecundity and fertility of adults *Callosobruchus chinensis* (L.) irradiated either as mature pupae or l-day-old adults decreased with the increase of the dose, complete sterility was obtained at 80 Gray in the l-day-old adults. Gill and Pajne [7] stated that males of *Callosobruchus chinensis* (Linneaus) irradiated as pupae with doses of 30 Gray inducing 92% sterility and 35 Gray inducing 98.7% sterility. My study suggests that the pulse beetle was relatively radiosensitive when compared with other species of stored product beetle. Jonson [8] reported that irradiated adults of the dried fruit beetle, *Carpophilus hemipterus* with 338 Gray produced no progeny.The sterile dose given by Abdel-Baky [9] for male of *Lasioderma serricorne* was 200 Gray. As a group, beetles are more radiosensitive than lepidopterous species. Rananavare *et al.* [10] found that exposure of virgin males of potato tubers warm, *Phthorimoea operculella* at 450 Gray produced permanent sterility. Study of Boshra and Hasaballa [11] on adults of *Polodia interpunctella* showed that the sterile dose for males was 650 Gray while it was 500 Gray when both sexes were treated and mated together.

Table (2) shows that when the sterilizing dose (120 Gray) divided into 2 or 3 equal fractions at 5 h time intervals, between each fraction did not change the complete sterility, there was no clear effect on fecundity and adult longevity. Similar results were obtained by Ducoff *et al.* [12] on *Tribolium confusum* and Ahmed [4] on *Callosobruchus maculatus*. They reported that recovery in fertility was not observed by dividing the sterilizing dose. Investigation of Brower [13] on *Plodia interpunctella* showed that by fractionating the sterilizing dose, the somatic or physiological damage might be minimized but the genetic damage causing sterility might remain the same. Study of La Chance and Graham [14] on males of *Musca domestica, Oncopeltus fasciatus, Anagasta kuhniella* and *Heliothis virescens,* showed that no reduction in the frequency of lethal mutations induced in any species when the acute dose was fractionated into 2 equal fractions eseparated by 8 h.

Table 2

Effects of sterilizing dose fractionation on egg production, egg hatch and longevity of *C. chinensis* treated as newly emerged males

No. of Fractions*	Dose In	Av. No. of Eggs/ o	% Egg Hatch	Av. Longevity (days)
		Io x Uo		
Control		58.6	86.3	7.00**
1	120	32.2	0	6.3
2	60	33.8	0	6.9
3	40	30.0	0	7.5
		Uo x i o		
Control		61.4	88.0	5.9***
1	120	26.0	0	4.7
2	60	22.5	0	6.1
3	40	19.80	0	6.3

Sretilizing dose for males and females 120 Gray

* 5 hours between each fraction

** Male longevity

*** Female longevity

Data presented in Table (3) shows, that when males were treated with substerilizing doses (40 and 80 Gray) and confined with unirradiated males and females at the ratio l:1:I (Io : Uo : Uo) gave rise to 52.1 and 43.0 percent hatch, respectively. As the sterile to fertile flooding ratio was increased from 1 : 1 to 10 : 1, the percentage of egg hatch was decreased to 20.6 and 9.0% with the above mentioned doses and flooding ratios. When males were irradiated at 120 Gray, the sterilizing dose, and confined with the unirradiated adults at the ratios 1:1:1 and 10:1:1 (Io : Uo : Uo), percent egg hatch was decreased from 85.2% for the control to 45.2 and 4.4% with the ratios 1:1:1 and 10:1:1, respectively. The competitiveness values of irradiated males increased with the increasing ratio of irradiated to unirradiated males at all doses level used. These values were within the limits considered fully competitive [3]. These results agreed with those obtained by Tilton *et al.* [15] on Trogoderma glabrum, they showed that males irradiated as adults with sterile dose 250 Gray were fully competitive with unirradiated males when released at high flooding ratios. Ahmed *et al.* [16, 17] on *Callosobruchus maculatus,* pointed out that even large decreases in competitiveness can be nullified by small increases in the required flooding ratio, when males treated as adults with the sterilizing dose (150 Gray) and confined together with untreated males and females at the ratio 15:1:1 caused 96.5% infertility in the resulting eggs. Ahmed and Younes [18] on *Callosobruchus maculatus* showed that males irradiated as l-day-old adults with gamma-ray doses

Table 3

Competitiveness value of *C. chinensis* males irradiated with sterile and substerile doses of gama rays.

Dose (Gray)	Ratio Io : Uo : Uo	% Egg Hatch	Competitiveness Value (C.V.)
	0 : 1 : 1	86.2	----
	1 : 0 : 1	15.7	----
40	1 : 1 : 1	52.1	0.99
	5 : 1 : 1	27.0	1.04
	10 : 1 : 1	20.6	1.34
	0 : 1 : 1	88.8	----
	1 : 0 : 1	3.5	----
80	1 : 1 : 1	43.0	1.16
	5 : 1 : 1	15.0	1.28
	10 : 1 : 1	9.0	1.45
	0 : 1 : 1	85.2	----
	1 : 0 : 1	0.0	----
120	1 : 1 : 1	45.0	0.88
	5 : 1 : 1	14.0	1.02
	10 : 1 : 1	4.4	1.84

I = Irradiated U = Unirradiated

of 75, 100 or 150 Gray, a sterilizing dose, were fully competitive with unirradiated males at most ratios. As the sterile to fertile flooding ratio was increased to 5:1 or to 10:1, the percentage of egg infertility was increased to 86.36 and 95.25%, respectively. Thus, the competitiveness value increased for 1.02 to 1.12 and 1.80 as the ratio increased from 1:1 to 5:1 and 10:1, respectively. Hussain and Lmura [6] reported that the sterilizd males of *Callosobruchus chinensis* irradiated as 1-day-old adults at 80 Gray were capable of competing sexually with untreated ones, the percentage of egg hatch decreased as the ratios of 9:1 and 15:1. Study of Abdel-Baky and Ibrahim [19] on *Corcyra cephalonica,* showed that increasing the competition ratio in favour of irradiated male from 1:1 to 5:1 gave more reduction in egg hatch, the competitiveness value was quite high among the irradiated males at any dose tested (150, 250 and 450 Gray). Investigation of Rananavar *et al.* [20] on *Phthorimoea operculella* showed that reduction in progeny production in one generation was 76 percent when sterile males were released.

Gill and Pajni [7] reported that sterile males of *Callosobruchus chinensis* (Linneaus) irradiated as pupae with 35 Gray when confined with normal males and females stopped further developed in 66.2 percent eggs laid. Further egg development decreased appreciably with increasing sterile male ratios, resulting in almost total suppression at a ratio of 8:1:1. The competitiveness values showed that sterile male were fully competitive with normal males at all the ratios.

Ahmed *et al.* [21] on *Plodia interpunctella,* reported that males sterilized at 600 Gray were capable of competing sexually with non-irradiated males at highest ratio used (25:1:1), increasing the percentages infertility until reaching 96.3% at the ratio 25:1:1.

Table (4) shows that replacing normal males by irradiated ones in normal population decreased percent egg hatch. Percent hatchability decreased from 86.0 in the first 2 days, to 30.8% after replacing normal males by sterile ones, in the second 2 days. These results indicated that sterilized males were able to negate previous insemination by a normal male. Also, insemination by normal males could partly overcome insemination by irradiated males, as the percent egg hatch increased from Zero to 35.0. The present results showed that sterile males are effective in reducing fertility and that their sperms can compete with those of unirradiated ones.

Table 4

Sperm activity of *C. chinensis* males irradtiated with sterile dose (120 Gray).

	1st Two Days		2nd Two Days	
Combination	Av. No. of Eggs/5o	% Hatch	Av. No. of Eggs/5o	% Hatch
Ia	150	86.0	82	30.8
Ib	162	85.8	125	86.6
IIa	101	00.0	133	35.0
IIb	123	00.0	85	00.0

Ia = U females x U males replaced by I males.

Ib = U females x U males replaced by U males.

IIa = U females x I males replaced by U males.

IIb = U females x I males replaced by I males.

Recognizable sperm inactivation seems to occur in insects at doses beyond those needed to induce 99% dominant lethality [22].

Similar results were obtained by El-Badry *et al.* [23] on *Spodoptera exigua* and Pair *et al.* [24] on *Heliothis virescens.* They showed that replacing normal males by sterile ones decreased egg hatchability. Younes [25] on *Cadra cautella* reported that replacing unirradiated males by irradiated ones (550 Gray) decreased the percentage of egg hatch from 86.84 in first 3 days in case of normal males to 33.27 in the 3 days following their replacement by irradiated ones, on the other hand, insemination by unirradiated males increased percentage of egg hatch from Zero to 36.63%. Ahmed *et al.* [17] on *Callosobruchus maculatus* showed that percent hatchability decreased from 88.9 in the first 3 days to 17.4 after replacing normal males by sterile males (sterile dose 150 Gray), in the second 3 days. Also, insemination by normal males could approximately nullify insemination by irradiated males as the egg hatchability increased from Zero to 47.8%. Haiba [2] on *Callosobruchus chinensis,* found that males irradiated as fully grown pupae with the sterile dose 70 Gray were more effective in decreasing percent hatchability from 88.23 to 32.44 when normal males were replaced by sterile males. Similar results were obtained by Abdel-Baky, [26] on *Ephestia kuhniella* males irradiated as full grown pupae with sterile dose 450 Gray and Boshra [27] on sterile males of *Plodia interpunctella* irradiated as full grown pupae at 600 Gray. Boshra and Hasaballa [11] on *Plodia interpunctella* showed that sperms of irradiated males with sterile dose 650 Gray could compete with sperms of normal males percent hatchability decreased from 86.4 in the first 2 days to 28.6 when normal males were replaced by sterile ones in the second 2 days. On the other hand, Jaynes and Godwin [28] found that females of *Pissodes strobi* mated with males receiving 100 and 200 Gray produced no viable off spring, whereas females previously receiving the same dose produced more viable off spring at the higher doses, indicating in activation of the irradiated sperm.

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